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(54) Biodegradable hydrogel matrices for the controlled release of pharmacologically active agents.

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Description

Technical Field

This invention relates generally to drug delivery systems, and more particularly relates to biodegradable hydrogel matrices useful in the controlled release of pharmacologically active agents.

Background

The last decade has seen rapid development in the area of drug delivery. In particular, a number of drug delivery systems have been developed to effect the controlled release of pharmacologically active agents. For a general overview of the art, reference may be had, inter alia, to R. Baker, Controlled Release of Biologically Active Agents, New York: John Wiley & Sons 1987. EP-A-0 224 987 discloses drug delivery systems based on hyaluronan or derivatives thereof. US-A-4,357,259 provides a method for incorporating water soluble therapeutic agents in albumin microspheres.

One area of research has been in the use of "hydrogels", or water-swellable polymeric matrices, in drug delivery systems. See, for example, P.I. Lee, J. Controlled Release 2:277-288 (1985). Hydrogels are network polymers which can absorb a substantial amount of water to form elastic gels. The release of pharmacologically active agents "loaded" into such gels typically involves both absorption of water and desorption of the agent via a swelling-controlled diffusion mechanism.

A significant drawback in the use of hydrogels, however, and one that has substantially hindered the use of hydrogels in drug delivery systems, is that such formulations are generally not biodegradable. Thus, drug delivery devices formulated with hydrogels typically have to be removed after subcutaneous or intramuscular application or cannot be used at all if direct introduction into the blood stream is necessary. Thus, it would be advantageous to use hydrogels which could be degraded after application in the body without causing toxic or other adverse reactions.

Only a few types of biodegradable hydrogels have been described. These have been based on proteins (e.g., using albumin microspheres, as described in S.S. Davis et al., J. Controlled Release 4:293-303 (1987)) or on poly(α amino acids), as described in H.R. Dickinson et al., J. Biomed Mater. Res. 15: 577-589 and 591-603 (1981)). Even these formulations, however, have proved problematic with regard to biocompatibility.

Collagen matrices, including collagen-mucopolysaccharide matrices, have been prepared and used for wound healing applications and in laminated membranes useful as synthetic skin. See, e.g., U.S. Patent Nos. 4,060,081, 4,280,954 and 4,418,691 to Yannas et al. and 4,485,096 to Bell. These collagen matrices, however, would have limited if any utility in drug delivery as they are not "blood compatible". That is, the properties of these matrices that enable wound healing--e.g., by facilitating clotting--teach against their use in drug delivery systems.

The inventor herein has now discovered a biodegradable hydrogel which has significantly enhanced biocompatibility in that (1) blood compatibility is substantially improved, (2) immunogenicity is minimized, and (3) the hydrogel is enzymatically degraded to endogenous, nontoxic compounds. The process for making the novel hydrogel represents a further advance over the art in that, during synthesis, one can carefully control factors such as hydrophilicity, charge and degree of cross-linking. By varying the composition of the hydrogel as it is made, one can control the uptake of a particular drug, the degradation kinetics of the hydrogel formulation and the overall timed-release profile.

Disclosure of the Invention

A drug delivery system comprising:

(a) a biodegradable hydrogel matrix comprising a protein, a polysaccharide, and a cross-linking agent providing network linkage therebetween, wherein the weight ratio of polysaccharide to protein in the matrix is in the range of 10:90 to 90:10 and wherein the cross-linking agent forms an amide bond or Schiff-base with the protein and the polysaccharide; and

(b) a drug contained within the matrix.

The invention also encompasses a method of making such a drug delivery system, comprising dissolving the aforementioned components in an aqueous medium, cross-linking the components to provide a three-dimensional network, and loading a selected drug, in solution or in liquid form, into the matrix. The composition of the hydrogel formed may be varied during synthesis so as to alter hydrophilicity, charge and degree of cross-linking.

As the systems of the present invention are blood- and tissue-compatible, they may be used to deliver a variety of drugs according to any number of modes of administration, e.g., oral, parenteral, or the like.

As noted above, a primary advantage of the novel hydrogels is in their enhanced biocompatibility. The use of polysaccharides or mucopolysaccharides (especially heparins) in the formulations is believed to enhance blood compatibility and significantly reduce activation of the complement system. Furthermore, as the polymeric components of the hydrogel are endogenous, the products of enzymatic degradation are endogenous as well.

Brief Description of the Figures

Figure 1 outlines the general reaction scheme of Example 1.

Figure 2 illustrates in flow chart form the method of preparing albumin-heparin microspheres as described in Example 2.

Figure 3 illustrates in flow chart form a modified method of preparing albumin-heparin microspheres as described in Example 2.

Figures 4 and 5 are scanning electron micrographs of albumin-heparin and albumin microspheres synthesized according to the method described in Example 2 for preparing chemically stabilized microspheres.

Figure 6 is a graph illustrating the swelling behavior of albumin-heparin microspheres prepared as described in Example 2.

Figures 7 and 8 illustrate in bar graph form similar swelling studies carried out in PBS buffer solutions of varying ionic strengths, as described in Example 3.

Figure 9 graphically represents the results of further swelling studies carried out to assess the effect of pH, also as described in Example 3.

Figure 10 is a graph illustrating the in vitro release profile from protein-loaded hydrogels prepared as described in Example 4.

Figures 11 and 12 are graphs illustrating the effect of hydrogel composition on the release of lysozyme, as described in Example 5.

Modes for Carrying Out the Invention

The drug delivery systems of the present invention are formed by cross-linking a polysaccharide or a mucopolysaccharide with a protein and loading a drug, in solution or in liquid form, into the hydrogel matrices so provided. The hydrogel matrices can be prepared using different ratios of polysaccharide or mucopolysaccharide to protein and can be produced in various sizes and geometries. Upon incorporation of the selected drug as will be described, the hydrogel can be swollen to various extents depending on the composition of the gel as well as on pH, temperature, and the electrolyte concentration of the loading medium. This permits the incorporation of different types and classes of drugs, including low molecular weight drugs, peptides and proteins. After exposure of the drug-containing hydrogel to the physiological environment, i.e., to blood or tissue, drugs will be released gradually. The release rate, like the loading parameters, will depend on the composition of the gel, the degree of cross-linking, any surface treatment of the components (e.g., to increase or decrease their hydrophilicity, charge, degradation kinetics), the type of drug used, and the geometry of the hydrogel body.

By "hydrogel" as used herein is meant a water-swellable, three-dimensional network of macromolecules held together by covalent cross-links. (These covalent cross-links are sometimes referred to herein as providing a "network linkage" within the macromolecular structure.) Upon placement in an aqueous environment, these networks swell to the extent allowed by the degree of cross-linking.

By the term "pharmacologically active agent" or "drug" as used herein is meant any chemical material or compound suitable for administration which induces a desired systemic or local effect. In general, this includes therapeutic agents in all of the major therapeutic areas. By "effective" amount of a pharmacologically active agent or drug is meant a nontoxic but sufficient amount of a compound to provide the desired systemic or local effect.

By a drug or pharmacologically active agent in "liquid form", as used herein, is intended a liquid drug, i.e., neat, or a drug dissolved or dispersed in a pharmacologically compatible carrier. By drug "contained within" a hydrogel matrix is meant a drug dispersed or dissolved therein.

By "protein", as used herein, is meant both full-length proteins and polypeptide fragments, which in either case may be native, recombinantly produced, or chemically synthesized.

"Polysaccharides", as used herein, are intended to include both polysaccharides and mucopolysaccharides.

Examples of suitable polysaccharides include heparin, fractionated heparins (e.g., on an AT-III column), heparan, heparan sulfate, chondroitin sulfate, and dextran. In general, the polysaccharides or mucopolysaccharides useful in forming the hydrogels of the invention are those described in U.S. Patent No. 4,060,081 to Yannas et al., cited supra. Heparin or heparin analogs are preferable because the compounds are strong anticoagulants and biodegradable by heparinases and amylases. In addition, using heparin or heparin analogs, i.e., compounds which are structurally related to heparin and which provide the same or similar degree of biocompatibility, appears to reduce immunogenicity and, because the compounds are highly charged, aqueous swelling is high, facilitating drug loading and release.

The protein component of the hydrogel may be, as noted above, either a full-length protein or a polypeptide fragment. It may be in native form, recombinantly produced or chemically synthesized. This protein component may also be a mixture of full-length proteins and/or fragments. Typically, the protein is selected from the group consisting of albumin, casein, fibrinogen, γ-globulin, hemoglobin, ferritin and elastin. This list is intended to be illustrative and not in any way limiting. For example, the protein component of the hydrogel may also be a synthetic polypeptide, e.g., a poly (α-amino acid) such as polyaspartic or polyglutamic acid.

Albumin is preferred as the protein component of the matrix as it is an endogenous material biodegradable in blood by proteolytic enzymes, in tissue by proteolytic enzymes associated with macrophage activity, and in different organs by phagocytosis, i.e., by the action of cells of the reticuloendothelial system (RES). Furthermore, albumin prevents adhesion of thrombocytes and is nontoxic and nonpyrogenic.

As noted above, a primary advantage of the invention lies in the fact that both the protein and polysaccharide components of the hydrogel are endogenous, biocompatible materials. This substantially reduces the likelihood of immunogenicity and, further, ensures that the products of biodegradation are also biocompatible materials.

The weight ratio of polysaccharide or mucopolysaccharide to protein within the hydrogel matrix is quite variable, and is typically within the range of 10:90 to 90:10. More preferably, the range is 10:90 to 60:40. The selected ratio affects drug loading, degradation kinetics, and the overall timed release profile. Thus, by varying the relative amounts of the protein and polysaccharide components in the hydrogel, one can, to a large extent, control the aforementioned factors.

In forming the novel hydrogels, one of several cross-linking methods may be used:

(1) The polysaccharide or mucopolysaccharide and the protein may be dissolved in an aqueous medium, followed by addition of an amide bond-forming cross-linking agent. A preferred cross-linking agent for this process is a carbodiimide, and a particularly preferred cross-linking agent here is the water-soluble carbodiimide N-(3-dimethyl-aminopropyl)-N-ethylcarbodiimide (EDC). In this method, the cross-linking agent is added to an aqueous solution of the polysaccharide and protein, at an acidic pH and a temperature of 0°C to 50°C, preferably 4°C to 37°C, and allowed to react for up to 48 hrs, preferably up to 24 hrs. The hydrogel so formed is then isolated, typically by centrifugation, and washed with a suitable solvent to remove uncoupled material.

(2) A mixture of the selected polysaccharide or mucopolysaccharide and protein is treated with a cross-linking agent having at least two aldehyde groups, thus forming Schiff-base bonds between the components. These bonds are then reduced with an appropriate reduction agent to give stable carbon-nitrogen bonds. A particularly preferred cross-linking agent in this procedure is glutaraldehyde, while a particularly preferred reduction agent is $NaCNBH_3$. The hydrogel matrix is isolated and purified as described above.

Prior to cross-linking, if desired, the polysaccharide component, e.g., heparin, can be partially de-N-sulfated via hydrolysis of $N\text{-}HSO_3$ groups to increase the number of free amine moieties available for cross-linking.

(3) The carboxylic and/or hydroxyl groups of a polysaccharide or mucopolysaccharide present in quaternary ammonium salt form--e.g., with Triton-B™ are preactivated by treatment with carbonyldiimidazole in a nonaqueous medium, e.g., formamide. This is followed by reaction with saccharine and subsequent reaction with the protein in an aqueous medium. Reaction time and temperature are the same as in (1) above.

(4) A conjugate of a polysaccharide or a mucopolysaccharide with a protein may be prepared as described in U.S. Patent No. 4,526,714 to Feijen et al. As described in that patent, conjugates of albumin and heparin may be prepared using EDC as the coupling agent.

The degree of cross-linking in the hydrogel, like the composition of the hydrogel itself, affects the degradation kinetics, loading, and overall timed release profile of the matrix. That is, a higher degree of cross-linking will generally result in slower degradation and release, while a lower degree of cross-linking will give faster degradation and release.

The hydrogel so formed is loaded with a selected drug by immersion in a solution containing the drug. Typically, hydrogels (albumin microspheres, for instance) are loaded by carrying out the crosslinking process in the presence of the drug. Alternatively, some hydrogels are loaded by immersion in a solution of the drug. Alternatively, some hydrogels are loaded by immersion in a solution of the drug in organic solvent(s), followed by evaporation of the organic solvent(s) after loading. The hydrogels of the present invention enable one to dispense with the use of organic solvents, and eliminate the possibility of contamination of the hydrogel with organic residues. That is, with the present method, the hydrogels may be loaded in an aqueous phase instead of in an organic solvent. With the present method, the hydrogels (microspheres) may be loaded in an aqueous phase drug solution after the hydrogel has been prepared and purified by washing.

The degree of drug loading is to a large extent dependent on the ionic strength of the aqueous system. In matrices formed from the ionically charged heparin (or analogs) and proteins, the degree of swelling increases significantly with decreasing ionic strength in the surrounding medium. Temperature may also be used to vary the degree of drug loading, as typically one will obtain a greater degree of drug loading at elevated temperatures due to higher swelling and drug solubility.

Another variable which influences the degree of drug loading is pH. Depending on the polysaccharide and protein used, changing the pH alters the degree of ionization, which will affect the swelling behavior of the gel and allow further flexibility in drug loading.

After equilibration, the loaded gels are dried in

vacuo under ambient conditions, and stored.

A wide variety of drugs may be incorporated into the hydrogel matrices, including low molecular weight drugs like hormones, cytostatic agents and antibiotics, peptides and high molecular weight drugs like proteins, enzymes and anticoagulants (such as heparin). Virtually any drug may be loaded into the hydrogel matrices, providing that considerations such as surface charge, size, geometry and hydrophilicity are taken into account. For example, incorporation and release of a high molecular weight drug will typically require a hydrogel having a generally lower degree of cross-linking. The release of a charged drug will be strongly influenced by the charge and charge density available in the hydrogel as well as by the ionic strength of the surrounding media.

The rate of drug release from the matrices can also be influenced by post-treatment of the hydrogel formulations. For example, heparin concentration at the hydrogel surface can be increased by reaction of the formulated hydrogels with activated heparin (i.e., heparin reacted with carbonyldiimidazole and saccharine) or with heparin containing one aldehyde group per molecule. A high concentration of heparin at the hydrogel surface will form an extra "barrier" for positively charged drugs at physiological pH values. Another way of accomplishing the same result is to treat the hydrogels with positively charged macromolecular compounds like protamine sulfate, polylysine, or like polymers. Still a further way of varying hydrogel permeability is to treat the surfaces with biodegradable block copolymers containing hydrophilic and hydrophobic blocks. The hydrophilic block can be a positively charged polymer like polylysine (which is able to covalently bind to the negatively charged heparin), while the hydrophobic block can be a biodegradable poly(α-amino acid) like poly(L-alanine), poly(L-leucine) or similar polymers.

It should be noted that several mechanisms are involved in the rate and extent of drug release. In the case of very high molecular weight drugs, the rate of release will be more dependent on the rate of hydrogel biodegradation. With lower molecular weight drugs, drug release will be more dominated by diffusion. In either case, depending on the hydrogel components selected, ionic exchange can also play a major role in the overall release profile. This is particularly true in applicants' preferred embodiment in which the hydrogel matrices have a substantial degree of ionic charge, e.g., matrices formed from ionically charged proteins (e.g., albumin) and heparin analogs.

The hydrogel matrices can be formed into capsules, tablets, films, microspheres, or the like. The compositions formulated using the hydrogel matrices can include conventional pharmaceutical carriers or excipients, adjuvants, etc. Matrices in the form of discs, slabs or cylinders can be used as implants, while microspheres can be applied as subcutaneous, intramuscular, intravenous or intra-arterial injectables. The size of the hydrogel bodies can be selected so as to direct ultimate placement. That is, depending on size, intravenously introduced microspheres may be physically trapped in the capillary beds of the lungs (size > 7 μm), phagocytosed by cells of the RES system (size > 100 nm) which will locate the particles mainly in the liver and spleen, or may become lodged at extracellular sites (size < 100 nm).

The following examples illustrate the invention.

Example 1

Preparation of a Biodegradable Hydrogel

Heparin (400 mg, 0.036 mmole) was added to 750 ml double distilled water. Human serum albumin ("HSA", 550 mg, 0.0085 mmole) was added to 1.0 ml double distilled water, and both solutions were kept at 4°C to dissolve overnight. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide ("EDC"), 94 mg, was then added to 250 ml double distilled water and dissolved at 4°C. The heparin solution, along with 1 ml of the albumin solution and a stir bar, was placed in a 2 ml polyethylene-polypropylene syringe of which the top had been cut off. A plunger was placed on the syringe and the solutions were thoroughly mixed. The EDC solution was added and the mixture was mixed again. All steps were carried out at 4°C.

After 24 hours, the resulting gel was removed from the syringe by swelling the syringe in toluene, and the gel was then equilibrated with phosphate buffered saline ("PBS") to remove uncoupled material.

Figure 1 outlines the general reaction scheme for this synthesis.

Example 2

(That part of this Example as well as of the reaction scheme of Figure 2 relating to the "denaturation stabilised" and "heat stabilised" microspheres does not fall within the subject matter of the present invention.

Preparation of Cross-linked Microspheres

Albumin-heparin microspheres were synthesized according to the reaction scheme shown in Figure 2. Pure olive oil (250 ml) was added to a flat-bottomed 400 ml beaker. A motor-driven double-bladed stirring bar was then submerged about two-thirds of the way into the oil. After stirring the oil for 30 minutes at 1500 rpm, 0.8 ml of an aqueous solution of albumin and heparin (~4:1, w/w) was added to the stirring oil with a 20 gauge syringe. The mixture was then stirred for 15 minutes. A solution of EDC in water (112 mgs/ml) was then added dropwise with a syringe, and the mix-

ture was stirred overnight. The microspheres (designated "chemically stabilized" in Figure 2) were isolated by centrifuging at 1000 rpm for 10 minutes and were subsequently vacuum filtered using a Teflon filter (pore size 0.45 microns) and washed with ether. The beads were then lyophilized and placed under vacuum overnight.

Other possibilities for obtaining the albumin-heparin microspheres are also outlined in Figure 2. "Denaturation stabilized" microspheres are prepared as described above, except that no cross-linking agent is used. "Heat-stabilized" microspheres are also prepared in the absence of a cross-linking agent, but at a temperature of about 100-170°C, typically about 130°C.

A modified synthesis scheme for preparing albumin-heparin microspheres, using a water-in-oil emulsion, is shown in the reaction scheme of Figure 3. In this method, 2.00 g Pluronic F-68 (a trademark of BASF Wyandotte Corp., Wyandotte, MI, for a high molecular weight polyoxyalkylene ether) was dissolved in 8.0 ml $CHCl_3$ in a 20 ml glass scintillation vial. Albumin (100 mg) and heparin (50.0 mg) were dissolved in 500 µl water and then added to the surfactant solution to form an emulsion. An EDC solution (24.0 mg/100µl) was injected into the emulsion and the mixture was stirred overnight. Isolation of the microspheres was carried out as described previously. All steps were carried out at 4°C.

Figures 4 and 5 are scanning electron micrographs of albumin-heparin and albumin microspheres, respectively, which were synthesized according to the method for preparing "chemically stabilized" microspheres as outlined above. In the case of the albumin microspheres, the procedure outlined above was followed except that heparin was omitted.

Example 3

Swelling Behavior of Albumin-Heparin Hydrogels

Swelling behavior of the albumin-heparin microspheres prepared as in the previous example (using varying amounts of heparin) was examined as follows.

a. The microspheres were placed in PBS buffer solution, pH 7.4, at 22°C, and the uptake of the buffer solution was monitored. As may be concluded from the graph shown in Figure 6, uptake of the buffer solution increased with heparin content. Thus, to be able to "load" more drug into the hydrogel matrices, heparin content should be correspondingly increased.

b. Swelling studies were also carried out in PBS buffer, pH 7.4, at varying ionic strengths. Equilibrium fractions of solution in the hydrogels were obtained for hydrogels of varying cross-link density. These values are presented in Figures 7 and 8. In pure water, where the shielding effects of counterions in solution can't mask the fixed charges within the hydrogels, swelling occurs until the hydrogel is mechanically very weak. These figures also demonstrate that the amount of "loading" that is possible is also dependent on the amount of cross-linking agent used as well as on the ionic strength of the solvent used.

c. Further swelling studies were done to evaluate the effect of pH. As above, the studies were carried out in PBS buffer at 22°C. Here, the ionic strength of the solution was maintained at 0.15. As illustrated in Figure 9, at low pH, the unreacted carboxylic acids (pKa about 4.2) are largely unionized, thus giving lower swelling. At higher pHs, swelling is correspondingly higher as well. This suggests that amines lose their protonation at higher pHs, thus reducing attractive electrostatic interaction.

Example 4

In Vitro Release of a Protein from Hydrogels

Chicken egg albumin (mol. wt. - 45,000) was dissolved in 4°C in double distilled water to make a final 10% (v/v) solution. Gels were then placed in 1 ml of these protein solutions for drug loading. When equilibrium was attained, the gels were subsequently dried at room temperature.

The dried protein loaded discs were then placed in 50 ml (1 disc per 50 ml) isotonic PBS buffer, pH = 7.40 w/ 0.1% sodium azide at room temperature. Samples of buffer solution were withdrawn at various intervals and assayed for chicken egg albumin. Release was quantified by UV spectroscopy (λmax=279.4 nm), and is shown in Figure 10.

Example 5

Effect of Composition on Macromolecular Release

The effect of albumin/heparin composition on macromolecular release was evaluated. Aqueous solutions containing either 28.6% and 17.1% albumin and heparin, respectively (i.e., a composition that is 5:3 wt./wt. albumin:heparin) or 34.3% and 11.4% albumin and heparin (i.e., a composition that is 6:2 wt./wt. albumin: heparin) were prepared. The pH was adjusted to 5.5 and the solutions cooled to 4°C. EDC was then added to both solutions to give 7.5% EDC, minimizing the exposure of the mixtures to air during EDC addition. The mixtures were then injected into film-shaped Mylar® molds which were refrigerated at 4°C overnight to allow in situ cross-linking of the albumin and heparin. The resultant gels were removed from the molds and discs were cut from the film with a cork bore. Final disc dimensions were 12.8 mm in

diameter by 1.9 mm in thickness.

Unincorporated albumin and heparin were then exhaustively extracted in isotonic PBS containing 0.1% sodium azide until no extractable components could be detected by UV spectroscopy (for albumin) or toluidine blue assays (for heparin).

Lysozyme, a 14,400 molecular weight protein, was loaded into the albumin-heparin gels by solution sorption techniques. Hydrogel discs were immersed in 20 ml of a 0.2% lysozyme aqueous solution, pH 7.3, and equilibrated for 50 hours. The lysozyme-loaded discs were removed from the loading solutions, dried with absorbent paper to remove surface-associated lysozyme, and dried at room temperature overnight.

Dried lysozyme-loaded discs were first weighed and then immersed in 50 ml (1 disc per 50 ml) PBS containing 0.1% sodium azide. Samples of the PBS were withdrawn at scheduled time points for lysozyme quantitation by UV spectroscopy (280.8 mm). Lysozyme release versus time (t) and $t^{1/2}$ is presented in Figures 11 and 12, respectively. From the release data, the lysozyme diffusion coefficients were determined to be 1.82×10^{-8} cm²/sec and 9.62×10^{-9} cm²/sec for 6:2 w/w and 5:3 w/w albumin-heparin hydrogels, respectively. As expected, then, a higher percentage of heparin in the hydrogel will decrease the release rate of the trapped pharmacologically active agent, presumably through ionic exchange interactions.

Claims

1. A drug delivery system comprising:
   (a) a biodegradable hydrogel matrix comprising a protein, a polysaccharide, and a cross-linking agent providing network linkage therebetween wherein the weight ratio of polysaccharide to protein in the matrix is in the range of 10:90 to 90:10 and wherein the cross-linking agent forms an amide bond or a Schiff-base with the protein and the polysaccharide; and
   (b) a drug contained within the matrix.

2. The drug delivery system according to claim 1, wherein the protein is selected from albumin, casein, fibrinogen, gamma-globulin, hemoglobin, ferritin, elastin and synthetic a-amino peptides.

3. The drug delivery system according to claim 2, wherein the protein is albumin.

4. The drug delivery system according to any one of the preceding claims wherein the polysaccharide is selected from heparin, heparin fragments, heparan, heparan sulfate, chondroitin sulfate, dextran and mixtures thereof.

5. The drug delivery system according to claim 4 wherein the polysaccharide is selected from heparin, heparin fragments, heparan and heparan sulfate.

6. The drug delivery system according to any one of the preceding claims wherein the ratio of polysaccharide to protein is in the range of 10:90 to 60:40.

7. The drug delivery system according to any one of the preceding claims wherein the cross-linking agent is an amide bond-forming agent.

8. The drug delivery system according to claim 7, wherein the amide bond-forming agent is a carbodiimide.

9. The drug delivery system of claim 8, wherein the carbodiimide is N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.

10. The drug delivery system according to any one of claims 1-6 wherein the cross-linking agent contains at least two aldehyde groups.

11. The drug delivery system according to claim 10, wherein the cross-linking agent is glutaraldehyde.

12. The drug delivery system according to any one of the preceding claims wherein the drug contained in the hydrogel matrix is either dissolved or dispersed therein.

13. The drug delivery system according to any one of the preceding claims wherein the drug is selected from proteins, enzymes, mucopolysaccharides, peptides, hormones, antibiotics and cytostatic agents.

14. A drug delivery system according to any one of the preceding claims in the form of a microsphere.

15. A substantially uniform film comprising a drug delivery system according to any one of claims 1 to 14.

16. A method for making a drug delivery system, comprising:
    dissolving a protein and a polysaccharide in an aqueous medium;
    cross-linking the protein and polysaccharide by forming amide bonds or Schiff-bases to provide a cross-linked hydrogel matrix; and loading a drug in solution or in liquid form into said hydrogel matrix to a predetermined degree.

17. A method according to claim 16 wherein the drug, protein, polysaccharide and cross-linking agent are as defined in any of claims 2-5, 7-11 or 13.

18. The method according to claims 16 or 17 wherein the cross-linking is effected by admixture of the protein and polysaccharide components with a cross-linking agent.

19. The method according to any one of claims 16-18 wherein the cross-linking agent contains at least two aldehyde groups, and wherein cross-linking is followed by reduction with $NaCNBH_3$.

20. The method according to claim 19, wherein the cross-linking agent is glutaraldehyde, followed by reduction with $NaCNBH_3$.

21. The method according to any one of claims 16-18 wherein the cross-linking is effected by:
   (a) initially dissolving the polysaccharide as a quaternary ammonium complex component in an organic reaction medium;
   (b) reacting the polysaccharide component with carbonyldiimidazole to give a preactivated polysaccharide compound;
   (c) further reacting the preactivated polysaccharide with saccharine, followed by
   (d) reacting the activated polysaccharide with a selected protein dissolved in the aqueous medium.

22. The method according to any one of claims 16-21 wherein the weight ratio of polysaccharide to protein is in the range of 10:90 to 90:10.

23. The method according to claim 22, wherein the ratio of polysaccharide to protein is in the range of 10:90 to 60:40.

24. The method according to any one of claims 16-23 wherein the degree of drug loading is controlled by the ratio of polysaccharide to protein and cross-linker in the hydrogel matrix.

25. The method according to any one of claims 16-23 wherein the degree of drug loading is controlled by temperature.

26. The method according to any one of claims 16-23 wherein the degree of drug loading is controlled by ionic strength.

27. The method according to any one of claims 16-23 wherein the degree of drug loading is controlled by pH.

28. A method of preparing microspheres useful in the controlled release of drugs, comprising:
   (a) preparing an aqueous solution of a selected protein and a polysaccharide, wherein the weight ratio of polysaccharide to protein in the solution is in the range of 10:90 to 90:10;
   (b) admixing the solution with oil to provide an emulsion, wherein the volume ratio of oil to protein solution is in the range of 1:1 to 500:1;
   (c) introducing a cross-linking agent into the emulsion so as to cross-link the protein and polysaccharide; and
   (d) isolating the microspheres so formed.

29. A method of preparing microspheres useful in the controlled release of drugs, comprising:
   (a) preparing an aqueous solution of a selected protein and a polysaccharide, wherein the weight ratio of polysaccharide to protein in the solution is in the range of 10:90 to 90:10;
   (b) introducing a cross-linking agent into the solution so as to cross-link the protein and polysaccharide, and to provide a cross-linked mixture, while
   (c) admixing the mixture with oil to provide an emulsion, wherein the volume ratio of oil to protein is in the range of 1:1 to 500:1; and finally
   (d) isolating the microspheres so formed.

30. The method according to claim 28 or 29 wherein after being isolated, the microspheres are heat-treated at a temperature in the range of 100°C to 170°C.

31. The method of any one of claims 28, 29 or 30, further comprising loading an effective amount of drug into the microspheres by immersing the microspheres in a solution of the drug.

32. A method of preparing films useful in the controlled release of drugs, comprising:
   (a) preparing an aqueous solution of a selected protein;
   (b) admixing the protein solution with a selected polysaccharide solution containing a cross-linking agent to cross-link the protein to the polysaccharide by forming amide bonds or Schiff bases;
   (c) casting the mixture on a substrate to give a substantially uniform film thereon; and
   (d) removing the film from the substrate.

33. The method of claim 32, further comprising loading an effective amount of drug into the films by immersing the films in a solution of drug.

Patentansprüche

1. Arzneimittelfreisetzungssystem, umfassend:
   a) eine biologisch abbaubare Hydrogelmatrix, umfassend ein Protein, ein Polysaccharid und ein Quervernetzungsagens, das zwischen diesen eine Quervernetzungsbindung ausbildet, wobei das Gewichtsverhältnis von Polysaccharid zu Protein in der Matrix im Bereich von 10:90 bis 90:10 liegt und wobei das Quervernetzungsagens eine Amidbindung oder eine Schiff'sche Base mit dem Protein oder Polysaccharid ausbildet und
   b) einen in der Matrix enthaltenen Arzneistoff.

2. Arzneimittelfreisetzungssystem nach Anspruch 1, bei dem das Protein ausgewählt ist aus Albumin, Casein, Fibrinogen, Gamma-Globulin, Hämoglobin, Ferritin, Elastin und synthetischen α-Aminopeptiden.

3. Arzneimittelfreisetzungssystem nach Anspruch 2, bei dem das Protein Albumin ist.

4. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche, bei dem das Polysaccharid ausgewählt ist aus Heparin, Heparinfragmenten, Heparan, Heparansulfat, Chondroitinsulfat, Dextran und Mischungen davon.

5. Arzneimittelfreisetzungssystem nach Anspruch 4, bei dem das Polysaccharid ausgewählt ist aus Heparin, Heparinfragmenten, Heparen und Heparansulfat.

6. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche, bei dem das Verhältnis von Polysaccharid zu Protein im Bereich von 10:90 bis 60:40 liegt.

7. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche, bei dem das Quervernetzungsagens ein Agens ist, das eine Amidbindung bildet.

8. Arzneimittelfreisetzungssystem nach Anspruch 7, bei dem das die Amidbindung bildende Agens ein Carbodiimid ist.

9. Arzneimittelfreisetzungssystem nach Anspruch 8, bei dem das Carbodiimid N-(3-Dimethylaminopropyl)-N'-Ethylcarbodiimid ist.

10. Arzneimittelfreisetzungssystem nach einem der Ansprüche 1 - 6, bei dem das Quervernetzungsagens mindestens zwei Aldehydgruppen enthält.

11. Arzneimittelfreisetzungssystem nach Anspruch 10, bei dem das Quervernetzungsagens Glutaraldehyd ist.

12. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche, bei dem der in der Hydrogelmatrix enthaltene Arzneistoff entweder gelöst oder dispergiert vorliegt.

13. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche, bei dem der Arzneistoff ausgewählt ist aus Proteinen, Enzymen, Mucopolysacchariden, Peptiden, Hormonen, Antibiotika und Zytostatika.

14. Arzneimittelfreisetzungssystem nach einem der vorhergehenden Ansprüche in Form von Mikrosphären.

15. Im wesentlichen gleichförmiger Film, umfassend ein Arzneimittelfreisetzungssystem nach einem der Ansprüche 1 - 14.

16. Verfahren zur Herstellung eines Arzneimittelfreisetzungssystems, umfassend Auflösen eines Proteins und eines Polysaccharids in einem wäßrigen Medium, Quervernetzen des Proteins und des Polysaccharids durch Bildung von Amidbindungen oder Schiff'schen Basen, um eine quervernetzte Hydrogelmatrix auszubilden, und Einbringen einer zuvor bestimmten Menge eines Arzneistoffes in Lösung oder in flüssiger Form in diese Matrix.

17. Verfahren nach Anspruch 16, bei dem man einen Arzneistoff, ein Protein, ein Polysaccharid und ein Quervernetzungssagens verwendet, wie sie in einem der Ansprüche 2 - 5, 7 - 11 oder 13 definiert sind.

18. Verfahren nach Anspruch 16 oder 17, bei dem die Quervernetzung durch Mischen der Protein- und Polysaccharidkomponenten mit einem Quervernetzungsagens bewirkt wird.

19. Verfahren nach einem der Ansprüche 16 - 18, bei dem das Quervernetzungsagens mindestens zwei Aldehydgruppen enthält und bei dem der Quervernetzung eine Reduktion mit $NaCNBH_3$ folgt.

20. Verfahren nach Anspruch 19, bei dem das Quervernetzungsagens Glutaraldehyd ist und wobei eine Reduktion mit $NaCNBH_3$ folgt.

21. Verfahren nach einem der Ansprüche 16 - 18, bei dem die Quervernetzung bewirkt wird durch:
   a) Zu Beginn Auflösen des Polysaccharids als eine quaternäre Ammoniumkomplex-Kompo-

nente in einem organischen Reaktionsmedium,
b) Umsetzen der Polysaccharidkomponente mit Carbonyldiimidazol zu einer voraktivierten Polysaccharidverbindung,
c) weiteres Umsetzen des voraktivierten Polysaccharids mit Sacharin, gefolgt von
d) Umsetzen des aktivierten Polysaccharids mit einem ausgewählten Protein, das in dem wäßrigen Medium gelöst ist.

22. Verfahren nach einem der Ansprüche 16 - 21, bei dem das Gewichtsverhältnis von Polysaccharid zu Protein im Bereich von 10:90 bis 90:10 liegt.

23. Verfahren nach Anspruch 22, bei dem das Verhältnis von Polysaccharid zu Protein im Bereich von 10:90 bis 60:40 liegt.

24. Verfahren nach einem der Ansprüche 16 - 23, bei dem das Ausmaß des Einbringens des Arzneistoffes durch das Verhältnis von Polysaccharid zu Protein und Quervernetzungsmittel in der Hydrogematrix gesteuert wird.

25. Verfahren nach einem der Ansprüche 16 - 23, bei dem das Ausmaß des Einbringens des Arzneistoffes durch die Temperatur gesteuert wird.

26. Verfahren nach einem der Ansprüche 16 - 23, bei dem des Ausmaß des Einbringens des Arzneistoffes durch die Ionenstärke gesteuert wird.

27. Verfahren nach einem der Ansprüche 16 - 23, bei dem das Ausmaß des Einbringens des Arzneistoffes durch den pH gesteuert wird.

28. Verfahren zur Herstellung von Mikrosphären, die zur kontrollierten Freisetzung von Arzneistoffen geeignet sind, umfassend:
a) Herstellen einer wäßrigen Lösung eines ausgewählten Proteins und eines Polysaccharids, wobei das Gewichtsverhältnis von Polysaccharid zu Protein in der Lösung im Bereich von 10:90 bis 90:10 liegt,
b) Mischen der Lösung mit einem Öl, um eine Emulsion auszubilden, wobei das Volumenverhältnis von Öl zu Proteinlösung im Bereich von 1:1 bis 500:1 liegt,
c) Einbringen eines Quervernetzungsagens in die Emulsion, um das Protein und das Polysaccharid querzuvernetzen und
d) Isolieren der so gebildeten Mikrosphären.

29. Verfahren zur Herstellung von Mikrosphären, die zur kontrollierten Freisetzung von Arzneistoffen geeignet sind, umfassend:
a) Herstellen einer wäßrigen Lösung eines ausgewählten Proteins und eines Polysaccharids, wobei das Gewichtsverhältnis von Polysaccharid zu Proteinlösung im Bereich von 10:90 bis 90:10 liegt,
b) Einbringen eines Quervernetzungsagens in die Lösung, um das Protein und das Polysaccharid querzuvernetzen und um eine quervernetzte Mischung auszubilden, während
c) die Mischung mit Öl gemischt wird, um eine Emulsion auszubilden, wobei das Volumenverhältnis von Öl zu Protein im Bereich von 1:1 bis 500:1 liegt und schließlich
d) Isolieren der so gebildeten Mikrosphären.

30. Verfahren nach Anspruch 28 oder 29, wobei dis Mikrosphären, nachdem sie isoliert wurden, bei einer Temperatur im Bereich von 100°C bis 170°C wärmebehandelt werden.

31. Verfahren nach einem der Ansprüche 28, 29 oder 30, das darüber hinaus das Einbringen einer Wirksamen Menge von Arzneistoffen in die Mikrosphären durch Eintauchen der Mikrosphären in eine Lösung des Arzneistoffes umfaßt.

32. Verfahren zur Herstellung von Filmen, die zur kontrollierten Freisetzung von Arzneistoffen verwendbar sind, umfassend:
a) Herstellen einer wäßrigen Lösung eines ausgewählten Proteins,
b) Mischen der Proteinlösung mit einer ausgewählten Polysaccharidlösung, die ein Quervernetzungsagens zum Quervernetzen von dem Protein und dem Polysaccharid unter Ausbildung einer Amidbindung oder Schiff'schen Basen enthält,
c) Gießen der Mischung auf ein Substrat unter Bildung eines im wesentlichen gleichförmigen Filmes und
d) Entfernen des Filmes vom Substrat.

33. Verfahren nach Anspruch 32, das darüber hinaus das Einbringen einer wirksamen Menge an Arzneistoff in den Film durch Eintauchen des Filmes in eine Lösung des Arzneistoffes umfaßt.

Revendications

1. Système de libération de drogue comprenant:
(a) une matrice d'hydrogel biodégradable comprenant une protéine, une polysaccharide et un agent réticulant qui produit un réseau de liaisons entre ces constituants, le rapport en poids du polysaccharide à la protéine étant, dans la matrice, dans la gamme de 10:90 à 90:10, et l'agent réticulant formant une liaison

amide ou une base de Schiff avec la protéine et le polysaccharide ;
(b) une drogue contenue dans la matrice.

2. Système de libération de drogue selon la revendication 1, dans lequel la protéine est choisie parmi l'albumine, la caséine, le fibrinogène, la gamma-globuline, l'hémoglobine, la ferritine, l'élastine et les α-amino-peptides de synthèse.

3. Système de libération de drogue selon la revendication 2, dans lequel la protéine est l'albumine.

4. Système de libération de drogue selon l'une quelconque des revendications précédentes, dans lequel le polysaccharide est choisi parmi l'héparine, les fragments d'héparine, l'héparane, l'héparane-sulfate, le chondroïtine-sulfate, le dextrane et leurs mélanges.

5. Système de libération de drogue selon la revendication 4, dans lequel le polysaccharide est choisi parmi l'héparine, les fragments d'héparine, l'héparane, l'héparane-sulfate.

6. Système de libération de drogue selon l'une quelconque des revendications précédentes, dans lequel le rapport en poids du polysaccharide à la protéine est dans la gamme de 10:90 à 60:40.

7. Système de libération de drogue selon l'une quelconque des revendications précédentes, dans lequel l'agent réticulant est un agent formateur de liaison amide.

8. Système de libération de drogue selon la revendication 7, dans lequel l'agent formateur de liaison amide est une carbodiimide.

9. Système de libération de drogue selon la revendication 8, dans lequel la carbodiimide est la N-(3-diméthylaminopropyl)-N'-éthylcarbodiimide.

10. Système de libération de drogue selon l'une quelconque des revendications 1-6, dans lequel l'agent réticulant contient au moins deux groupes aldéhyde.

11. Système de libération de drogue selon la revendication 10, dans lequel l'agent réticulant est le glutaraldéhyde.

12. Système de libération de drogue selon l'une quelconque des revendications précédentes, dans lequel la drogue contenue dans la matrice d'hydrogel est soit dissoute, soit dispersée dans celle-ci.

13. Système de libération de drogue selon l'une quelconque des revendications précédentes, dans lequel la drogue est choisie parmi les protéines, les enzymes, les mucopolysaccharides, les peptides, les hormones, les antibiotiques et les agents cytostatiques.

14. Système de libération de drogue selon l'une quelconque des revendications précédentes, sous la forme d'une microsphère.

15. Film essentiellement uniforme, comprenant un système de libération de drogue selon l'une quelconque des revendications 1 à 14.

16. Procédé d'obtention d'un système de libération de drogue, consistant à:

dissoudre une protéine et un polysaccharide dans un milieu aqueux ;

réticuler la protéine et le polysaccharide par formation de liaisons amide ou de bases de Schiff pour produire une matrice d'hydrogel réticulée ; et charger ladite matrice d'hydrogel d'une drogue en solution ou sous forme liquide, dans une quantité prédéterminée.

17. Procédé selon la revendication 16, dans lequel la drogue, la protéine, le polysaccharide et l'agent réticulant sont tels que définis dans l'une quelconque des revendications 2-5, 7-11 ou 13.

18. Procédé selon la revendication 16 ou 17, dans lequel la réticulation est réalisée en mélangeant les composants protéine et polysaccharide avec un agent réticulant.

19. Procédé selon l'une quelconque des revendications 16 à 18, dans lequel l'agent réticulant contient au moins deux groupes aldéhyde, et dans lequel la réticulation est suivie d'une étape de réduction à l'aide du $NaCNBH_3$.

20. Procédé selon la revendication 19, dans lequel l'agent réticulant est le glutaraldéhyde, la réticulation étant suivie d'une étape réduction à l'aide du $NaCNBH_3$.

21. Procédé selon l'une quelconque des revendications 16 à 18, dans lequel la réticulation est effectuée au moyen des étapes consistant à :
(a) solubiliser initialement un composant polysaccharide sous forme d'un complexe d'ammonium quaternaire dans un milieu réactionnel organique ;
(b) faire réagir le composant polysaccharide avec le carbonyldiimidazole pour obtenir un composé polysaccharide préactivé ;
(c) faire encore réagir le polysaccharide préactivé avec de la saccharine, puis

(d) faire réagir le polysaccharide activé avec une protéine sélectionnée dissoute dans un milieu aqueux.

22. Procédé selon l'une quelconque des revendications 16 à 21, dans lequel le rapport en poids du polysaccharide à la protéine est dans la gamme de 10:90 à 90:10.

23. Procédé selon la revendication 22, dans lequel le rapport en poids du polysaccharide à la protéine est dans la gamme de 10:90 à 60:40.

24. Procédé selon l'une quelconque des revendications 16 à 23, dans lequel la quantité de drogue introduite est réglée par le rapport du polysaccharide à la protéine et l'agent réticulant dans la matrice d'hydrogel.

25. Procédé selon l'une quelconque des revendications 16 à 23, dans lequel la quantité de drogue introduite est réglée par la température.

26. Procédé selon l'une quelconque des revendications 16 à 23, dans lequel la quantité de drogue introduite est réglée par la force ionique.

27. Procédé selon l'une quelconque des revendications 16 à 23, dans lequel la quantité de drogue introduite est réglée par le pH.

28. Procédé de préparation de microsphères utilisables dans la libération contrôlée de drogues, comprenant les étapes consistant à :

(a) préparer une solution aqueuse d'une protéine sélectionnée et d'un polysaccharide, le rapport en poids du polysaccharide à la protéine dans la solution étant dans la gamme de 10:90 à 90:10 ;
(b) mélanger la solution avec de l'huile pour donner une émulsion, dans laquelle le rapport en poids du polysaccharide à la protéine dans la solution est dans la gamme de 1:1 à 500:1.
(c) introduire dans l'émulsion un agent réticulant de façon à réticuler la protéine et le polysaccharide ; et
(d) isoler les microsphères ainsi formées.

29. Procédé de préparation de microsphères utilisables dans la libération contrôlée de drogues, comprenant les étapes consistant à :

(a) préparer une solution aqueuse d'une protéine sélectionnée et d'un polysaccharide, le rapport en poids du polysaccharide à la protéine dans la solution étant dans la gamme de 10:90 à 90:10 ;
(b) introduire dans la solution un agent réticulant de façon à réticuler la protéine et le polysaccharide, et produire un mélange réticulé ; et
(c) mélanger le mélange avec de l'huile pour donner une émulsion, dans laquelle le rapport en volume de l'huile à la protéine est dans la gamme de 1:1 à 500:1. ; et enfin,
(d) isoler les microsphères ainsi formées.

30. Procédé selon la revendication 28 ou 29, dans lequel les microsphères, après avoir été isolées, sont soumises à un traitement thermique à une température dans la gamme de 100°C à 170°C.

31. Procédé selon l'une quelconque des revendications 28, 29 ou 30, consistant en outre à charger les microsphères d'une quantité efficace d'une drogue, en les immergeant dans une solution de cette dernière.

32. Procédé de préparation de films utilisables dans la libération contrôlée de drogues, comprenant les étapes consistant à :

(a) préparer une solution aqueuse d'une protéine sélectionnée ;
(b) mélanger la solution de protéine avec une solution d'un polysaccharide sélectionné, contenant un agent réticulant destiné à réticuler la protéine avec le polysaccharide en formant des liaisons amide ou des base de Schiff ;
(c) couler le mélange sur un substrat pour obtenir sur celui-ci un film essentiellement uniforme ; et
(d) enlever le film du substrat.

33. Procédé selon la revendication 32, consistant en outre à charger les films d'une quantité efficace d'une drogue, en les immergeant dans une solution de cette dernière.

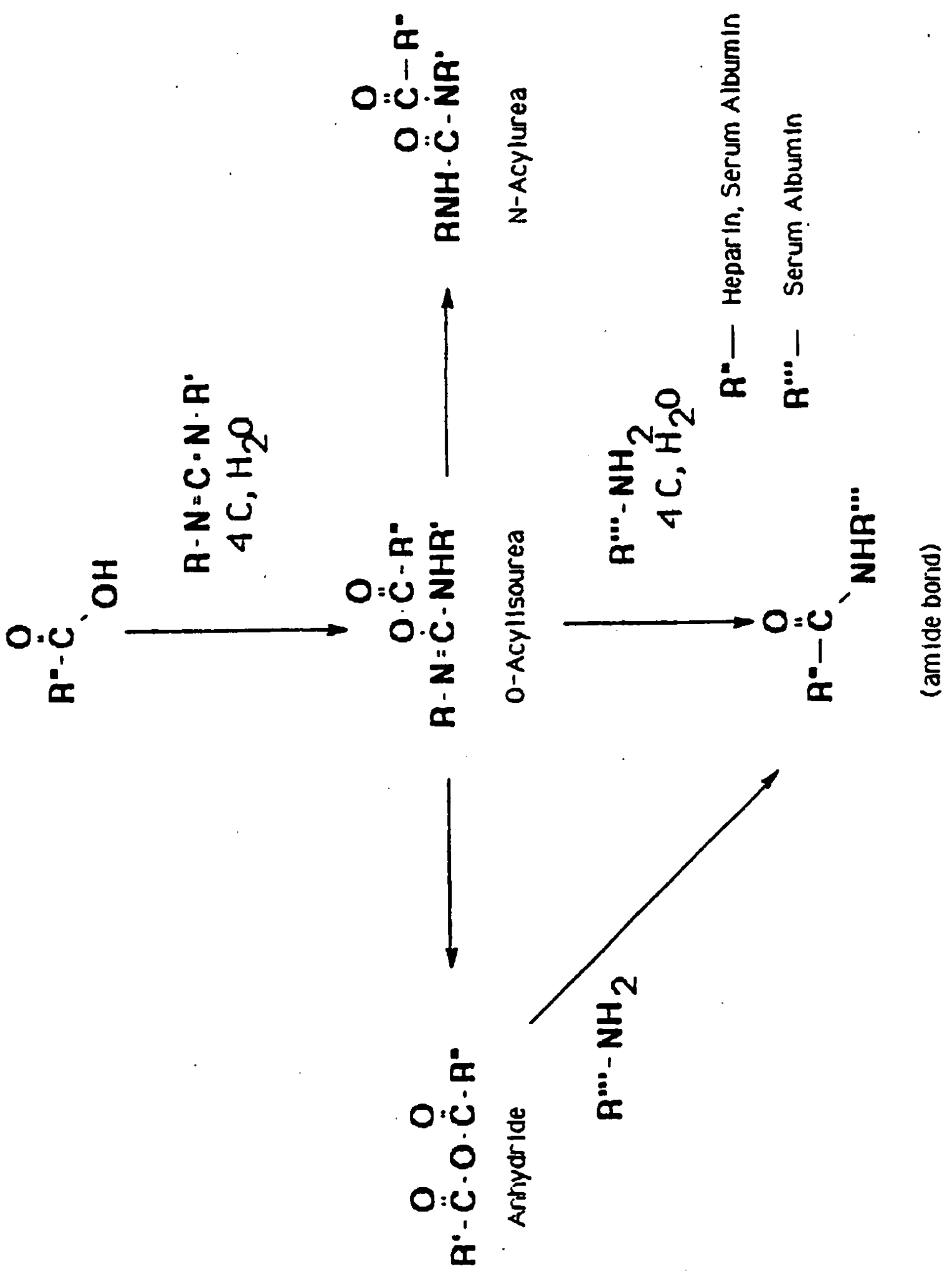
R-N=C-N-R'
4 C, H2O
O-Acylisourea
N-Acylurea
Anhydride
R'''-NH2
4 C, H2O
(amide bond)
R" — Heparin, Serum Albumin
R''' — Serum Albumin

Fig. 1

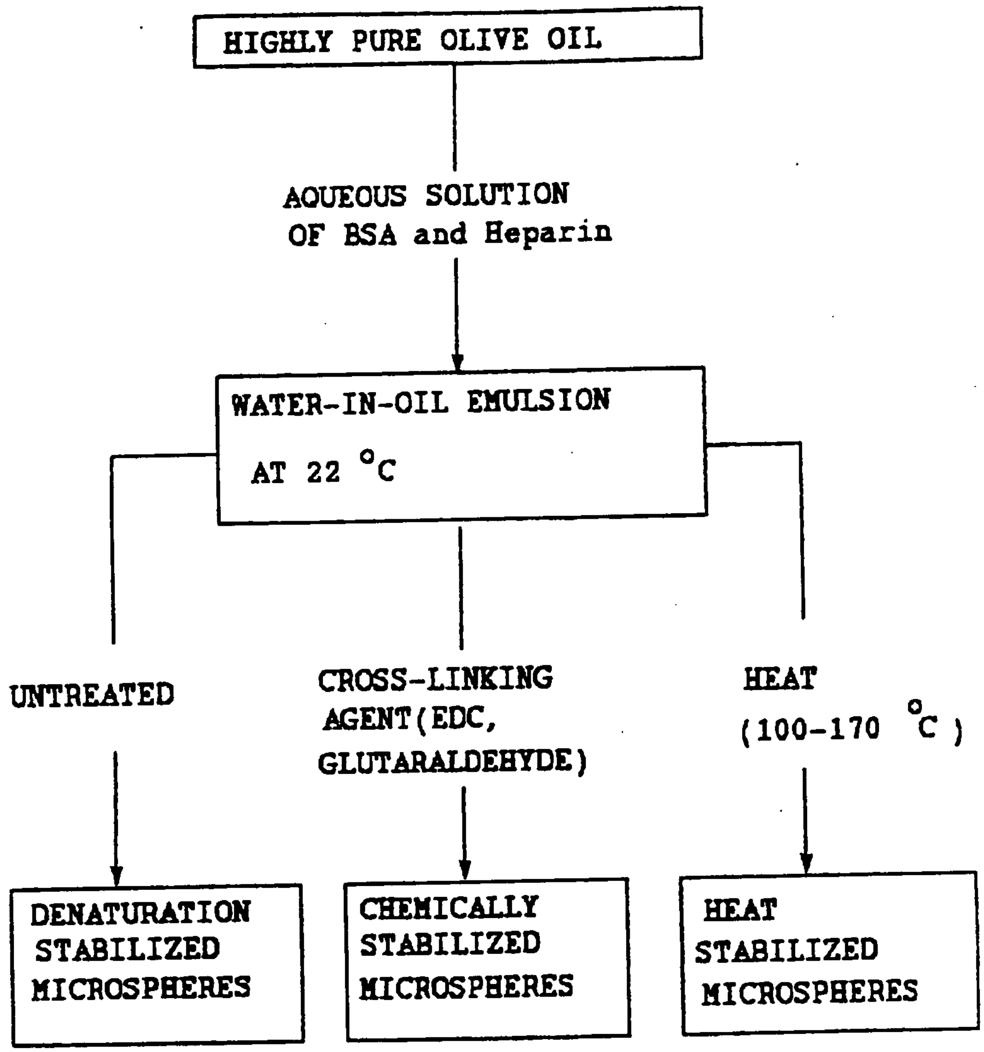
HIGHLY PURE OLIVE OIL
AQUEOUS SOLUTION OF BSA and Heparin
WATER-IN-OIL EMULSION AT 22 °C
UNTREATED
CROSS-LINKING AGENT(EDC, GLUTARALDEHYDE)
HEAT (100-170 °C)
DENATURATION STABILIZED MICROSPHERES
CHEMICALLY STABILIZED MICROSPHERES
HEAT STABILIZED MICROSPHERES

Fig. 2

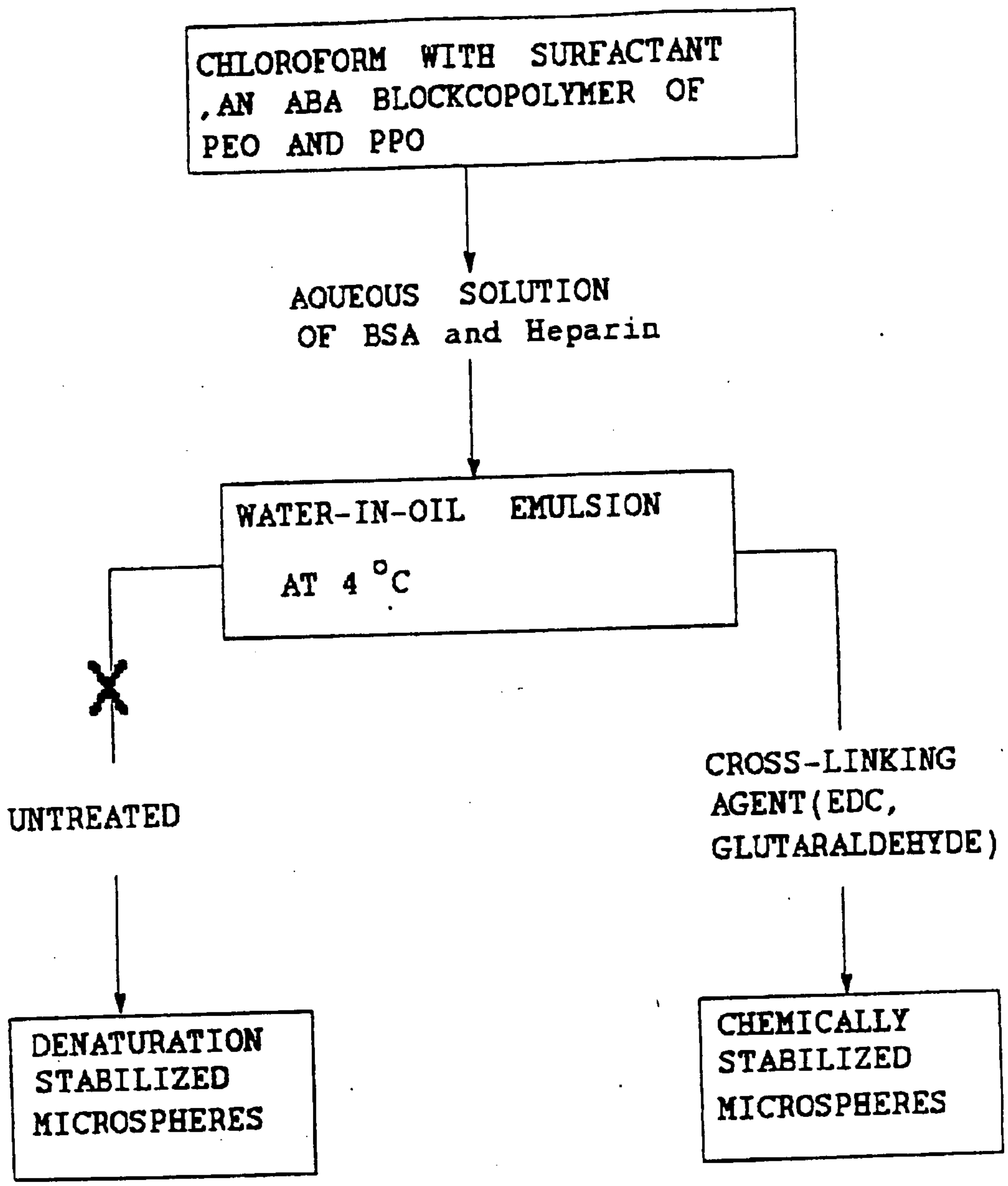
CHLOROFORM WITH SURFACTANT
,AN ABA BLOCKCOPOLYMER OF
PEO AND PPO
AQUEOUS SOLUTION
OF BSA and Heparin
WATER-IN-OIL EMULSION
AT 4 °C
UNTREATED
CROSS-LINKING
AGENT(EDC,
GLUTARALDEHYDE)
DENATURATION
STABILIZED
MICROSPHERES
CHEMICALLY
STABILIZED
MICROSPHERES

Fig. 3

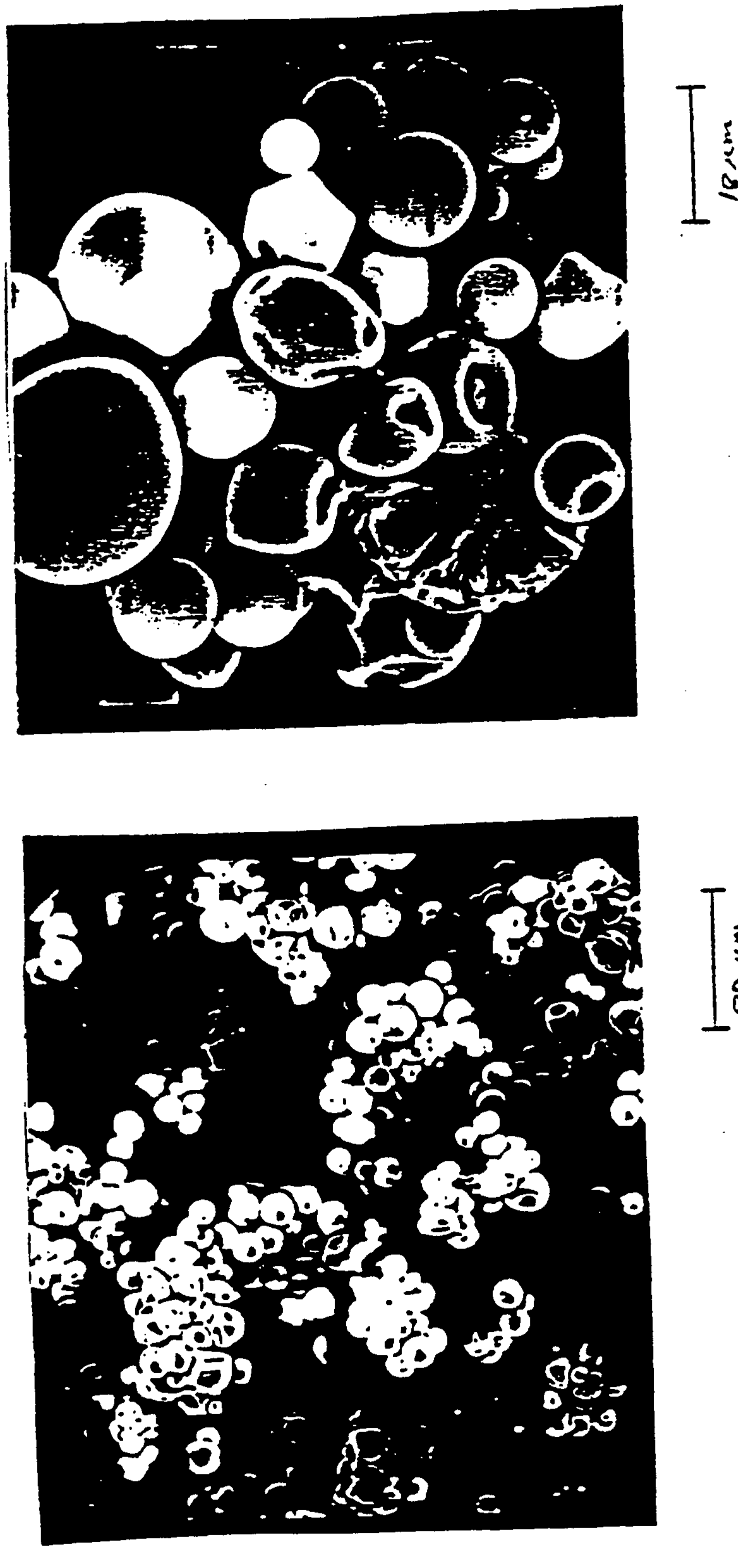

Fig. 4

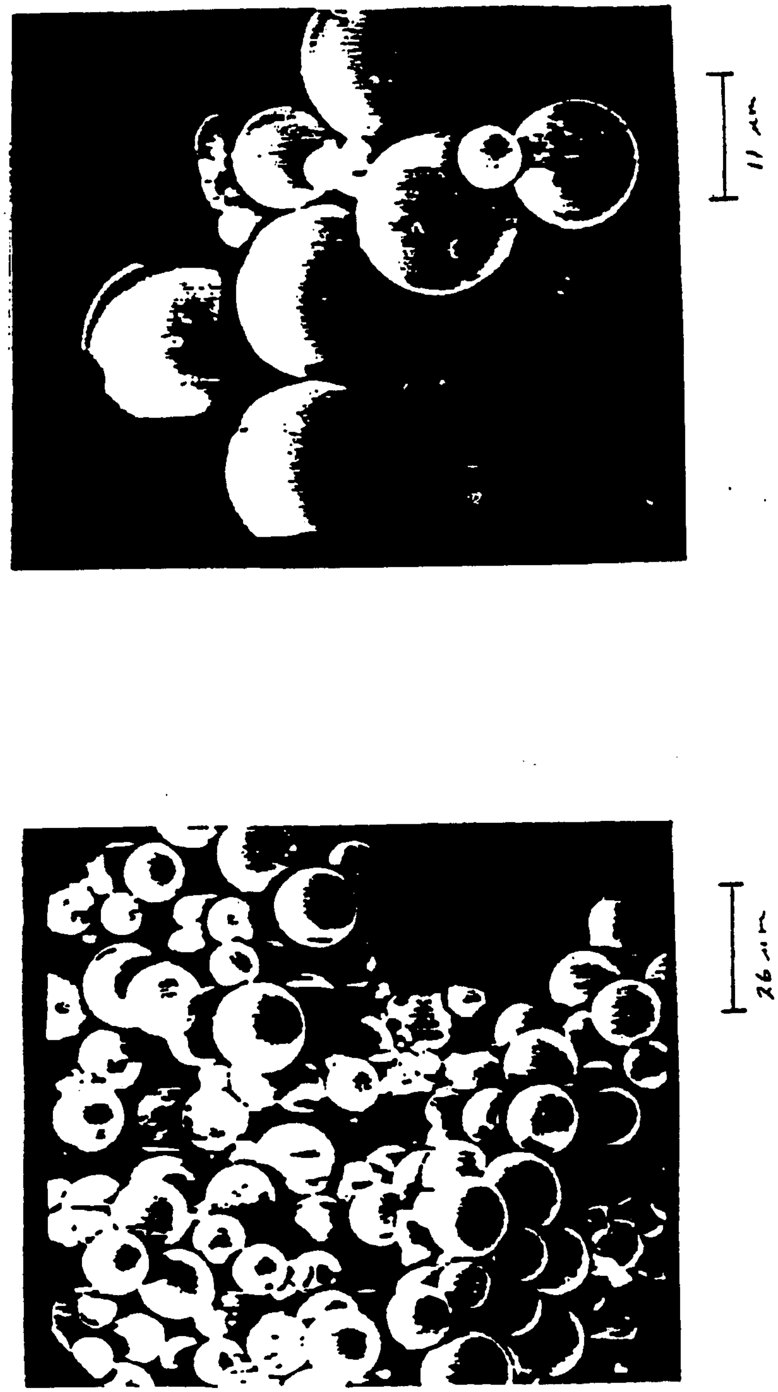

Fig. 5

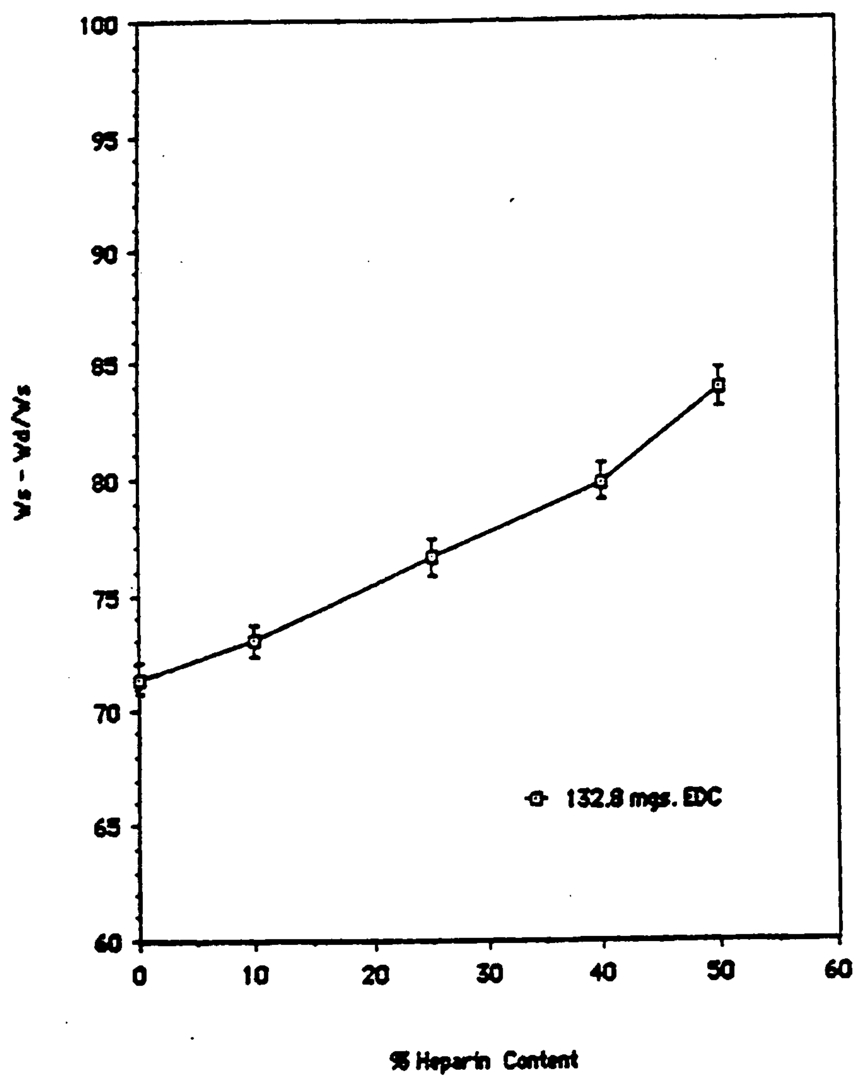
100
95
90
85
80
75
70
65
60
Ws - Wd/Ws
0
10
20
30
40
50
60
% Heparin Content
132.8 mgs. EDC

Fig. 6

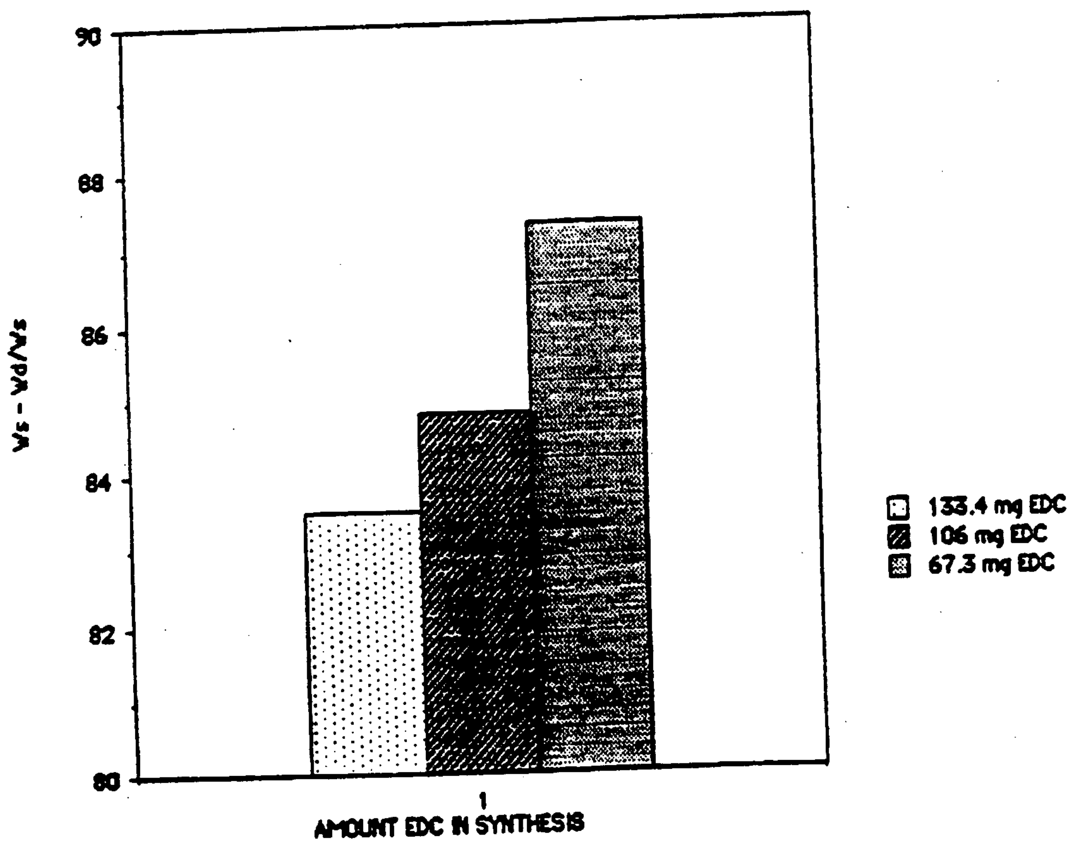
90
88
86
84
82
80
Vs - Vd/Vs
1
AMOUNT EDC IN SYNTHESIS
133.4 mg EDC
106 mg EDC
67.3 mg EDC

Fig. 7

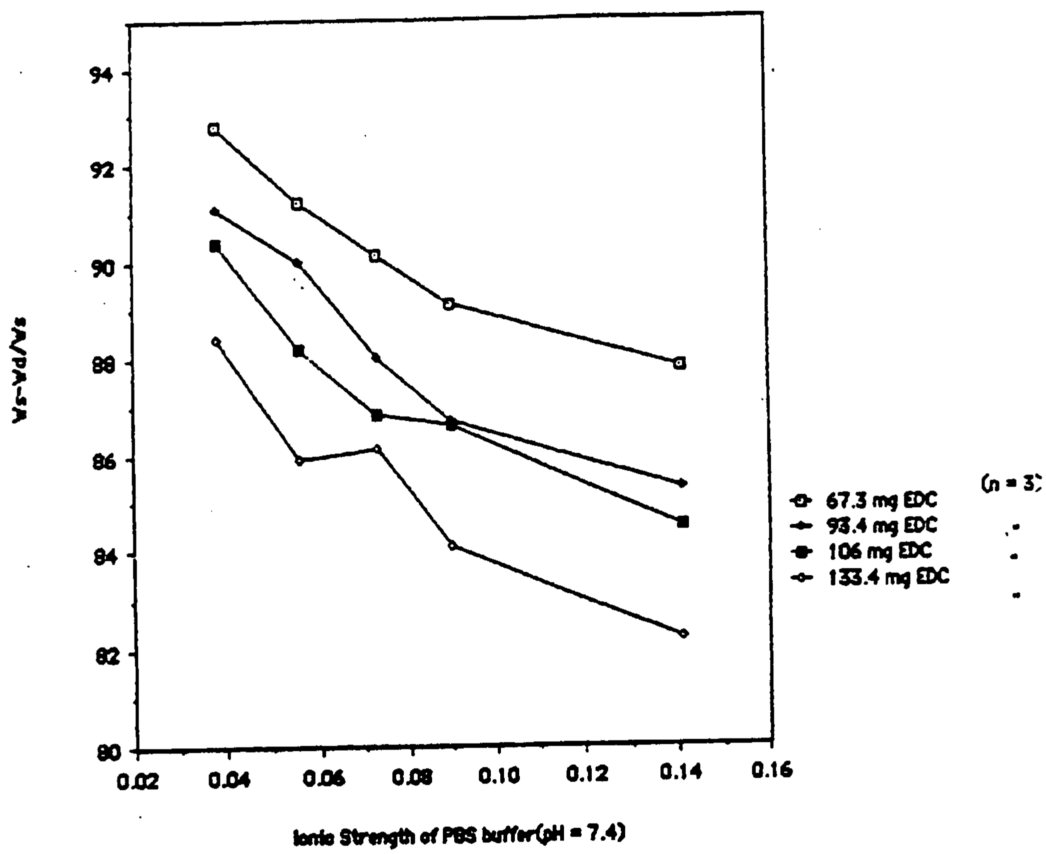
94
92
90
88
86
84
82
80
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
sA/PA-SA
Ionic Strength of PBS buffer(pH = 7.4)
67.3 mg EDC
93.4 mg EDC
106 mg EDC
133.4 mg EDC
(n = 3)

Fig. 8

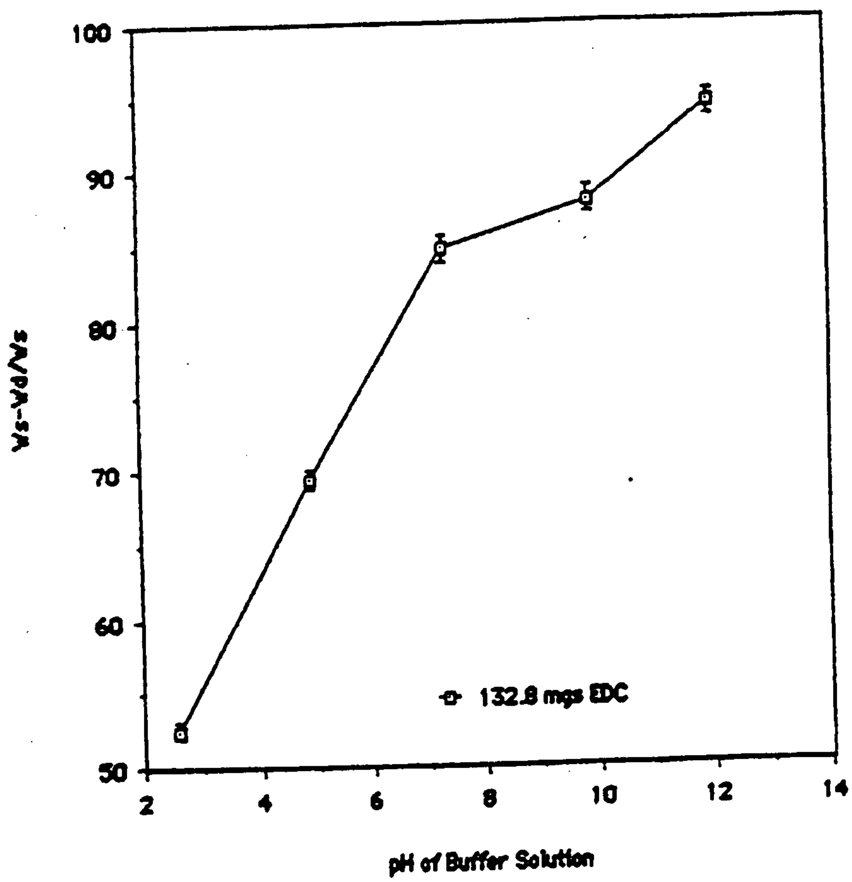
100
90
80
70
60
50
Vs-Vd/Vs
2
4
6
8
10
12
14
pH of Buffer Solution
132.8 mgs EDC

Fig. 9

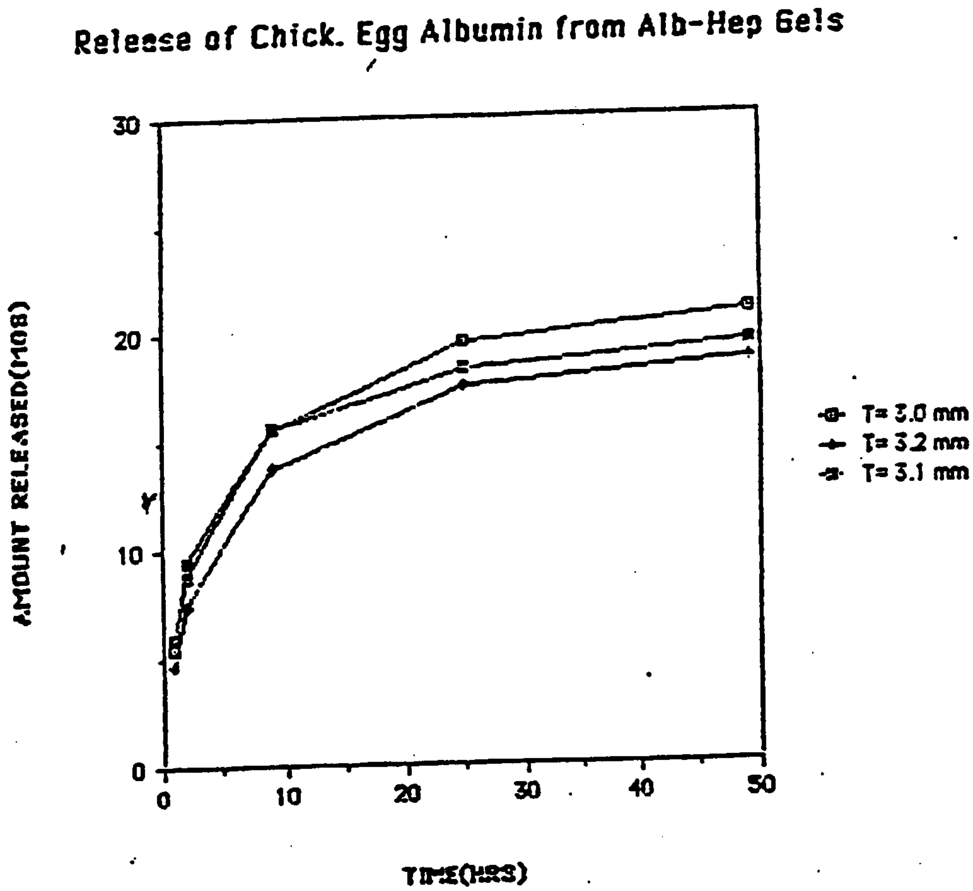
Release of Chick. Egg Albumin from Alb-Hep Gels
AMOUNT RELEASED(MOB)
TIME(HRS)
0
10
20
30
40
50
T= 3.0 mm
T= 3.2 mm
T= 3.1 mm

Fig. 10

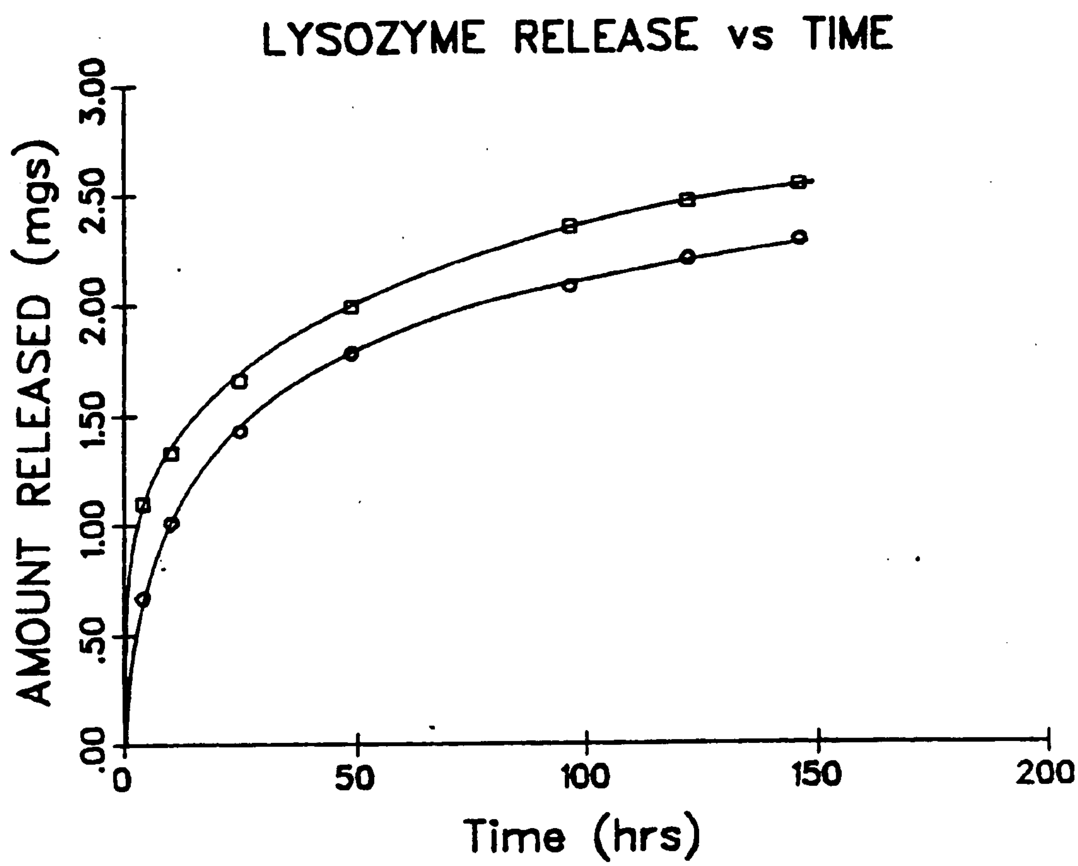

Fig. 11 Legend: Circles represent 6:2 alb-hep and squares represent 5:3 alb-hep.

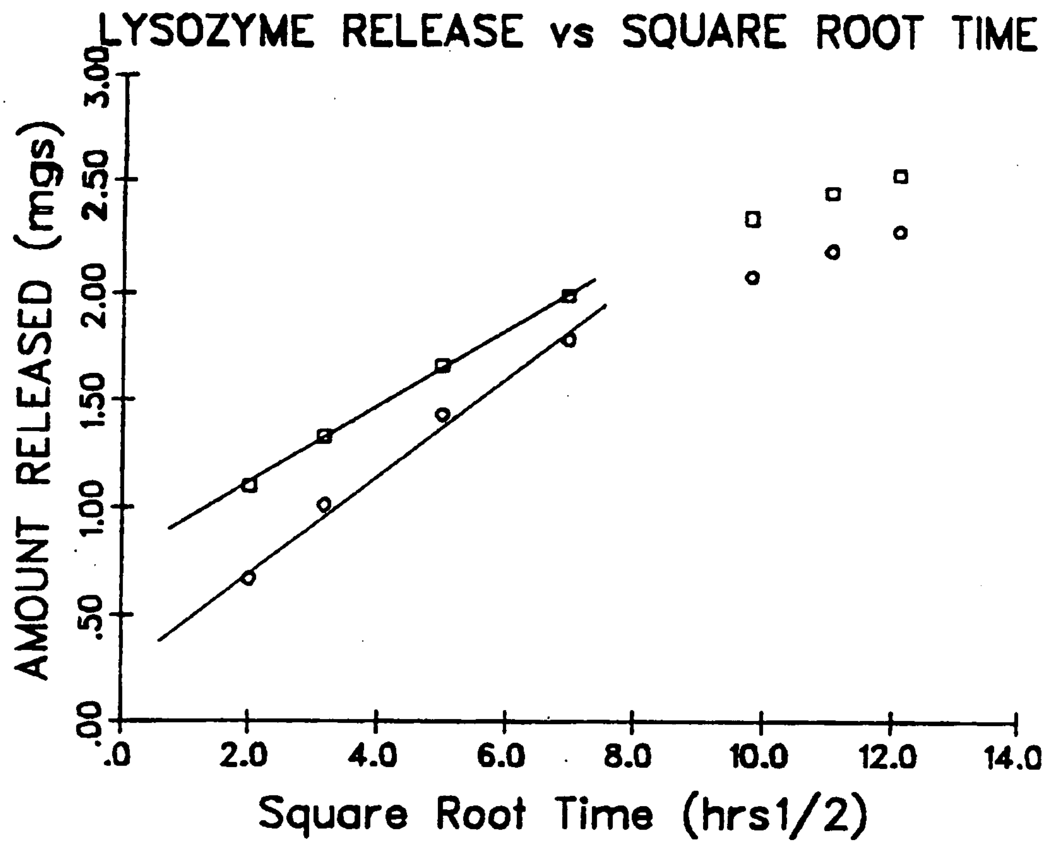

Fig. 12 Legend: Circles represent 6:2 alb-hep and squares represent 5:3 alb-hep.